Assignment¹ of the porcine FUS1 gene to SSC13q21→q22 by somatic cell and radiation hybrid panel mapping

K. Gatphayak, C. Knorr, and B. Brenig

Institute of Veterinary Medicine, Georg August University of Göttingen, Göttingen (Germany)

¹ To our knowledge this is the first time this gene has been mapped in swine.

Rationale and significance

FUS1 is a candidate tumor suppressor gene organized in a 370 kb region which contains a set of 19 genes. The human FUS1 genomic sequence spans 3.3 kb containing 3 exons (NM_007275; Lerman and Minna, 2000). Overexpression of the FUS1 gene leads to G₁ arrest and growth inhibition of lung cancer cells (Kondo et al., 2001). The human and the murine FUS1 genes were assigned to chromosome HSA3p21.3 and MMU9F1, respectively. The sequence characterization of a porcine PAC clone revealed the identical gene cluster for pigs and human with respect to genes FUS1–HYAL2–HYAL1–FUS2–HYAL3. The porcine HYAL3 gene has recently been mapped to SSC13q21→q22 by fluorescence in situ hybridization (FISH) and radiation hybrid panel analysis while the somatic cell hybrid panel analysis resulted in the localization of the gene to SSC13q42→(1/2q46) (Gatphayak et al., 2003). Considering the gene order in the cluster, the latter assignment is in accordance with the mapping of the porcine HYAL1 gene – which was provisionally called LUCA1 (for LUNg CAncer) – to SSC13q23→(1/2)q41 by Lahbib-Mansais et al. (1999) also using the somatic cell hybrid panel. Here we report the mapping of the porcine FUS1 gene by somatic cell and radiation hybrid panel and provide enhanced knowledge about the localization of the gene cluster.

Methods and materials

The porcine P1-derived artificial chromosome library developed at the Institute of Veterinary Medicine in Göttingen (Al-Bayati et al., 1999) was screened by PCR using primers specific for the human HYAL3 gene (Accession No: NM_003549). Sequencing of the isolated PAC clone and BLAST comparison (results not shown) revealed the existence of several genes – including FUS1 – on this clone. Physical mapping of FUS1 was performed by somatic cell hybrid panel (Yerle et al., 1996) as well as by radiation hybrid panel (Yerle et al., 1998) analysis. Primers FUS1-fw (5'-GCTCCATGTTCTATGACGAG) and FUS1-rev (5'-GTTCTCAATGGAAGCCACAC) were defined based on sequence information derived from exon 2 and the 3' UTR region of the porcine gene. A 439-bp PCR product was amplified under the following conditions: 95 °C for 3 min, followed by 34 cycles with 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min in the last cycle. Obtained RH panel results were analyzed using the INRA Toulouse web site (<http://imprh.toulouse.inra.fr>) and the results for the somatic cell hybrid panel were analyzed using <http://www.toulouse.inra.fr/lgc/pig/hybrid.htm>.

Results and discussion

Somatic cell hybrid panel

The somatic cell hybrid panel analysis of 27 hybrids resulted in the following vector: 00010 10000 01000 00000 01000 00. Statistical evaluations showed a significant correlation between the FUS1 gene and chromosome 13 of 0.99 (error risk about 0.1%, maximal correlation 0.85). The most probable region within SSC13 is q23→q(1/2 q41).

Radiation hybrid panel

The RH panel analysis resulted in the vector: 11011 00001 01000 11001 11000 01011 01000 00000 00010 10000 10000 10010 00010 00000 00001 00000 00000 00001 11000 01011 10101 00001 10110 100. The evaluation data (two point analysis) showed that the most significant linked markers are SSC24F05 and SW864 on SSC13 (17 cR and 29 cR distance, LOD-scores were 16.23 and 13.46, respectively). Multipoint

Supported by budgetary funds of the Institute of Veterinary Medicine.

Received 12 August 2003; manuscript accepted 22 August, 2003.

Corresponding author: Dr. Bertram Brenig, Institute of Veterinary Medicine
University of Göttingen, Gröner Landstrasse 2, D-37073 Göttingen (Germany)
telephone +49-551-393383, fax +49-551-393392; email: bbrenig@gwdg.de

analysis led to linkage group SW1400–SW864–SSC24F05–FUS1–SW1495–SW882–SW992–SW2459. SW1400 (Alexander et al., 1996) and the EST SSC24F05 (Jørgensen et al., 1997; Thomsen et al., 1998) have recently been mapped to SSC13q21→q22 and SW2459 as the terminal marker of the linkage group to SSC13q31 (Alexander et al., 1996). The most likely chromosomal position of FUS1 after RH panel mapping is therefore SSC13q21→q22.

In this report we mapped the FUS1 gene as a second gene from a porcine PAC clone originally screened for the characterization of the HYAL3 gene, which was mapped to SSC 13 by FISH, somatic cell and radiation hybrid panel analyses (Gatphayak et al. 2003). RH panel mapping of HYAL3 and FUS1 revealed for both genes the closest linkage to STS-markers SSC24F05 and SW864. The distance in cR (17) and the LOD score (16.23) with respect to SSC24F05 are identical for HYAL3 and FUS1. FUS1 is, however, more tightly linked to SW864 (29 cR, LOD score of 13.46) than HYAL3 is (33 cR, LOD score of 12.46). The lower-resolution somatic cell hybrid panel data indicate a more distal assignment of the gene cluster on chromosome 13 than the FISH and RH panel mapping: FUS1 to q23→(1/2q41) and HYAL3 to q42→(1/2q46). Our somatic cell hybrid panel results and those by Lahbib-Mansais et al. (1999) for LUCA1 (SSC13q23→[1/2q41]) are in agreement with the sequencing data indicating the gene order FUS1–HYAL2–HYAL1–FUS2–HYAL3. The correct localization of the gene group is, however, SSC13q21 as FISH and RH panel mapping assign the clone harboring the gene cluster to this position (Gatphayak et al., 2003).

References

- Al-Bayati KH, Duscher S, Kollers S, Rettenberger G, Fries R, Brenig B: Construction and characterization of a porcine P1-derived artificial chromosome (PAC) library covering 3.2 genome equivalents and cytogenetical assignment of six type I and type II loci. *Mammal Genome* 10:569–572 (1999).
- Alexander LJ, Troyer DL, Rohrer GA, Smith TPL, Schook LB, Beattie CW: Physical assignments of 68 porcine cosmid and lambda clones containing polymorphic microsatellites. *Mammal Genome* 7:368–372 (1996).
- Gatphayak K, Knorr C, Habermann F, Fries R, Brenig B: Assignment of the porcine hyaluronidase-3 (HYAL-3) gene to SSC13q21 by FISH and confirmation by hybrid panel analyses. *Cytogenet Genome Res* (in press).
- Jørgensen CB, Winterø AK, Fredholm M: Mapping of 22 expressed sequence tags isolated from a porcine small intestine cDNA library. *Mammal Genome* 8:423–427 (1997).
- Kondo M, Ji L, Kamibayashi C, Tomizawa Y, Randle D, Sekido Y, Yokota J, Kashuba V, Zabarovsky E, Kuzmin I, Lerman MI, Roth J, Minna JD: Overexpression of candidate tumor suppressor gene FUS1 isolated from the 3p21.3 homozygous deletion region leads to G1 arrest and growth inhibition of lung cancer cells. *Oncogene* 20:6258–62 (2001).
- Lahbib-Mansais Y, Dalias G, Milan D, Yerle M, Robic A, Gyapay G, Gellin J: A successful strategy for comparative mapping with human ESTs: 65 new region assignments in the pig. *Mammal Genome* 10:145–153 (1999).
- Lerman MI, Minna JD: The 630-kb lung cancer homologous deletion region on human chromosome 3p21.3: identification and evaluation of the resident candidate tumor suppressor genes. The International Lung Cancer Chromosome 3p21.3 Tumor Suppressor Gene Consortium. *Cancer Res* 60:6116–33 (2000).
- Thomsen PD, Winterø AK, Fredholm M: Chromosomal assignments of 19 porcine cDNA sequences by FISH. *Mammal Genome* 9:394–396 (1998).
- Yerle M, Echard G, Robic A, Mairal A, Dubut-Fontana C, Riquet J, Pinton P, Milan D, Lahbib-Mansais Y, Gellin J: A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. *Cytogenet Cell Genet* 73:194–202 (1996).
- Yerle M, Pinton P, Robic A, Alfonso A, Palvadeau Y, Celcros C, Hawken R, Alexander L, Beattie C, Schook L, Milan D, Gellin J: Construction of a whole genome radiation hybrid panel for high resolution gene mapping in pigs. *Cytogenet Cell Genet* 82:182–188 (1998).